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Amplification of the Proinflammatory Transcription Factor Cascade Increases with Severity of Uncontrolled Hemorrhage in Swine[1,2]

Susan I. Brundage, M.D., M.P.H.,*[,3] Martin A. Schreiber, M.D.,† John B. Holcomb, M.D.,‡ Nathan Zautke, B.S.,* Mary Ann Mastrangelo, M.S.,§ Xu Xq,§ Joe Macaitis, B.S.,‡ and David J. Tweardy, M.D.§

*Department of Surgery, Stanford University Medical Center, Palo Alto, California; †Department of Surgery, Oregon Health and Science University, Portland, Oregon; ‡U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas; and §Department of Medicine, Baylor College of Medicine, Houston, Texas

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Introduction. **Hypotension causes diffuse liver injury accompanied by increased local production of interleukin-6 (IL-6) in swine models of uncontrolled hemorrhagic shock (HS). IL-6 is transcriptionally upregulated by nuclear factor (NF)-κB and results in activation of signal transducer and activator of transcription-3 (Stat3) in a murine model of controlled HS. Our objectives were: 1) to determine if increased IL-6 production and NF-κB and Stat3 activation occurs in a swine model of uncontrolled HS, and 2) to assess whether or not levels of IL-6 mRNA and activity of NF-κB and Stat3 correlate with shock severity.**

Materials and methods. **Swine were assigned to four groups: 1) control animals ($n = 6$): no intervention, 2) sham operation ($n = 6$): celiotomy and splenectomy, 3) uncontrolled hemorrhagic shock (UHS) ($n = 6$): sham plus grade V vascular liver injury and resuscitation, 4) profound uncontrolled hemorrhagic shock (PUHS) ($n = 8$): UHS after dilutional hypothermia. Following euthanasia at 2 h, livers were harvested, total RNA isolated, and IL-6 mRNA levels quantified by Q-RT-PCR (ABI Prism 7700, Applied Biosystems International, Foster City, CA). Protein was extracted for measurement of NF-κB and Stat3 activity by electrophoretic mobility shift assay (EMSA).**

Results. **Compared to shams, IL-6 mRNA levels increased 4.5-fold in UHS and 90-fold in PUHS ($P < 0.001$). Compared with shams; NF-κB activity increased 2-fold in both UHS and PUHS ($P < 0.05$). Stat3 activity was equivalent (not significant) in UHS when compared with shams but increased 5.3-fold in PUHS. ($P < 0.05$).**

Conclusion. **These findings suggest that regional proinflammatory cytokine production results from and perpetuates a proinflammatory transcription factor cascade in a swine model of uncontrolled hemorrhagic shock and indicate that this process is proportional to the severity of shock.**

Key Words: **hemorrhage; shock, ischemia/reperfusion injury; trauma; interleukin-6; proinflammatory cytokines; inflammatory cascade; transcription factors; nuclear factor-κB; signal transducer and activator of transcription-3 (Stat3); signal transduction.**

INTRODUCTION

The majority of late deaths following severe trauma and hemorrhagic shock result from dysfunctional inflammation with subsequent multiorgan dysfunction syndrome (MODS) [1]. Our understanding of the molecular basis for shock-induced MODS is incomplete and largely based on controlled hemorrhagic shock protocols in rodents. Using these models, our laboratory and other investigators have demonstrated an essential role for IL-6 in post injury dysfunctional inflammation [2–6]. There have been few studies examining MODS in large animals subjected to uncontrolled hemorrhagic shock (UHS), a scenario that more closely approximates the clinical situation of hemorrhagic shock following trauma. Consequently, our under-

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[3] To whom correspondence should be addressed at the Department of Surgery, Stanford University Medical Center, 300 Pasteur Drive, Room H-3680, Stanford, CA 94305. E-mail: sbrundage@stanford.edu.

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standing of the molecular events contributing to post resuscitation dysfunctional inflammation in a model similar to the patient setting is limited.

Our group and others have demonstrated that dysfunctional inflammation in rodent models of controlled hemorrhagic shock (HS) is mediated; in part, through activation of latent transcription factors such as nuclear factor-κB (NF-κB) by reactive oxygen species and induced nitric oxide [7]. NF-κB binds to the promoter of variety of proinflammatory genes, including IL-6, resulting in their transcriptional activation. In turn, IL-6 activates signal transducer and activator of transcription (Stat)-3 in an autocrine and paracrine fashion leading to further amplification of the local inflammatory process.

The objectives of the studies summarized here were to determine whether increased IL-6 production and NF-κB and Stat3 activation occurs in a swine model of uncontrolled HS and to assess whether or not levels of IL-6 mRNA and activity of NF-κB and Stat3 correlate with shock severity.

MATERIALS AND METHODS

Animals and Protocol

All protocols were approved by the Animal Protocol Review Committee of Baylor College of Medicine and were performed according to the provisions of the Animal Welfare Act, PHS Animal Welfare Policy, the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the policies and procedures of Baylor College of Medicine. Three-month-old Yorkshire crossbred swine, weighing approximately 40 kg, were utilized. Animals were allowed access to water and commercial laboratory swine food *ad libitum* until 24 h prior to the procedures. Swine were assigned to four groups: 1) control animals ($n = 6$): no intervention; 2) sham operation ($n = 6$): celiotomy and splenectomy; 3) uncontrolled hemorrhagic shock (UHS) ($n = 6$): sham plus Grade V vascular liver injury and resuscitation; 4) profound uncontrolled hemorrhagic shock (PUHS) ($n = 8$): UHS after dilutional hypothermia. The groups are described in detail as follows:

Normal group. The control group of six animals were euthanized without performing any surgical procedure to obtain the baseline levels of IL-6 mRNA. NF-κB, and STAT-3 and the histology of nonoperated and nonhemorrhaged tissues.

Sham operated group. The sham group was given a preanesthetic intramuscular injection of glycopyrolate 0.01 mg/kg and Telazol 4 mg/kg. A 7.0-mm-diameter cuffed endotracheal tube was placed following induction of anesthesia. Anesthesia was maintained for the study period with 1–3% isoflurane in 0.5 FIO_2. Tidal volume was fixed at 10 ml/kg with a rate of 10 breaths per minute. Animals were placed in the supine position, the ventral cervical area and ventral abdomen clipped and surgically prepared with povidone iodine. An EKG monitor was secured. Vascular cutdowns were performed and polyethylene tubing placed in the left external jugular vein and left common carotid artery. The venous line was used for fluid resuscitation, whereas the arterial line was used for continuous blood pressure monitoring and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure, and heart rate were recorded and averaged every 10 s using a digital data collection system with a blood pressure analyzer (Micro-Med®, Louisville, KY). An esophageal stethoscope, gastric tube, and thermometer were inserted. A laparotomy was performed. A splenectomy was performed and the splenic hilum ligated with 0-silk suture. A splenectomy was necessary because the swine spleen is contractile and contains a variable amount of blood resulting in autoresuscitation of blood during hemorrhage studies, leading to a confounding variable; splenectomy removes this variable. The spleen was weighed and warm lactated Ringer's solution was infused at three times the weight to replace the blood sequestered in the spleen. A cystotomy was performed and a Foley catheter was placed. The abdominal wall was closed with towel clips and the animal was allowed to stabilize for 15 min. The starting temperature was standardized to 38°C. After stabilization, the abdomen was reopened and the liver was mobilized and manipulated. The peritoneal cavity was left open for the same time period required to clamp the liver in the hemorrhaged animals. The abdomen was then reapproximated with towel clips and the animal was kept under general anesthesia until euthanized at the completion of the 2-h study period. After euthanasia, the liver was collected and frozen in liquid nitrogen and placed in formalin for histologic analysis.

Uncontrolled hemorrhagic shock protocol. The UHS group ($n = 6$) underwent the same procedure as the sham operated animals with the addition of a Grade V liver injury during the laparotomy. During the laparotomy, preweighed laparotomy pads were placed in both peritoneal gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury, as defined by the American Association for the Surgery of Trauma Organ Injury Scale, was accomplished with a specially designed liver clamp. This Grade V liver injury has been described previously [8, 9]. The clamp was positioned in the middle of the liver placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. The injury was a vascular Grade V liver injury as opposed to a parenchymal crush injury. Blood loss was collected by suction. Bleeding was monitored for 15 min after injury. Blood loss was calculated, laparotomy pads were removed, and the abdomen were closed. Resuscitation with lactated Ringer's at 100 ml/min was instituted 15 min after liver injury, allowing the swine to reach a nadir blood pressure simulating the clinical trauma situation. Animals were resuscitated to their baseline mean arterial pressure (MAP). After completion of the 2-h study period, the abdomen was reopened and blood loss was determined. Blood samples were drawn at two time points. Baseline and final blood specimens were collected for complete blood count and liver enzymes. Tissues were collected as described in the sham operation. The site of liver harvested for examination was distant to the vascular injury. In addition, the liver was removed and examined to insure comparable vascular hepatic injuries.

Profound uncontrolled hemorrhagic shock protocol. Profound uncontrolled hemorrhagic shock (PUHS; $n = 8$): UHS plus packing preceded by dilutional hypothermia with a 60% isovolemic exchange transfusion with 5% albumin and cooling to 33°C; rendering the animal hypothermic with a dilutional coagulopathy. Sixty percent of the animals' blood volume was replaced with pharmaceutical grade 5% human albumin. The animals' temperature was standardized to an esophageal temperature of 33°C by lavaging the abdomen with cool lactated Ringer's. The animals were allowed to stabilize prior to performing the standardized Grade V liver injury. Immediately after the Grade V liver injury was performed, the abdomen was packed with laparotomy sponges to prevent exsanguination and fluid resuscitation initiated with lactated Ringer's solution. Blood samples were drawn at two time points. Baseline and final blood specimens were collected for prothombin times/partial thromboplastin times (PT/PTT), complete blood count, and liver enzymes. At the completion of the 2-h study period, the animals were euthanized, the liver inspected to ensure comparable vascular injuries, and tissues were harvested. Coagulopathy was confirmed by comparing mean baseline to final PT/PTT. Baseline prothombin time was measured at 13 s compared with final prothombin time of 34 s ($P < 0.002$). Baseline partial thromboplastin time was measured at 31 s compared with final partial thromboplastin time of 49 s ($P < 0.006$).

RNA Isolation and Quantitative Reverse Transcription-Polymerase Chain Reaction (Q-RT-PCR)

Liver was flash frozen in liquid nitrogen immediately upon harvest and stored at −80°C. Total RNA was extracted with commercially available Trizol Reagent ® (Life Technologies, GibcoBRL, Gaithersburg, MD). Total RNA (5 ng for 18S and 500 ng for IL-6 and G-CSF) was added to a reverse-transcriptase, first-strand cDNA synthesis reaction. A 20-μl reaction volume was subjected to reverse-transcriptase first-strand cDNA synthesis using the commercially available Taqman Multiscribe ® Reverse Transcriptase Kit (Applied Biosystems, Branchburg, NJ) under the following conditions: 10 min at 25°C, 30 min at 48°C, followed by inactivation of the enzyme at 95°C for 5 min (ABI Prism 7700 Thermocycler, Applied Biosystems, Branchburg, NJ).

Fifty μl of the first-strand cDNA synthesis was placed into a TaqMan PCR reaction in triplicate. Polymerase chain reaction (PCR) conditions were as follows: stage 1, 2 min at 50°C; stage 2, 10 min at 95°C; stage 3, 40 cycles of 15 s of melting at 95°C followed by DNA synthesis for 1 min at 60°C. The ABI Prism PE7700 was used as well as Taqman ® Universal Master Mix (Applied Biosystems).

PCR primers and probe for swine IL-6 were designed using the computer program Primer Express ® (Applied Biosystems) based upon the published swine sequences [10]. The forward primer for swine IL-6 was 5′-GCTGCTTCTGGTGATGGCTACT-3′, whereas the reverse was 5′-GGCATCACCTTTGGCATCTT-3′. The probe for IL-6 was located one nucleotide downstream of the 3′ end of the forward primer and was labeled at the 5′ end with 6FAM and at the 3′ end with the quencher TAMRA (6FAM-CCTTCCCTACCCCGGAAC-GCCT-TAMRA). A negative control for each set of PCR reactions contained sterile water instead of cDNA template. The cycle threshold of each triplicate determination was normalized by subtraction of the cycle threshold for its corresponding 18S cycle threshold (ΔCT). Each ΔCT was then calibrated by subtracting the mean normalized cycle threshold ΔCT of the six normal control organs ($\Delta\Delta$CT). The control animals sacrificed without operation were considered to have "normal" or constitutive levels of cytokine production. The level of mRNA for a given cytokine in the group of normal animals was arbitrarily assigned the value 1 and the fold change in cytokine mRNA levels in the sham and UHS groups compared to controls was calculated as $2^{-\Delta\Delta CT}$ as described in the User Bulletin 2, Perkin-Elmer Applied Biosystems.

Protein Isolation and EMSA Analysis

Liver was flash frozen in liquid nitrogen immediately upon harvest and stored at −80°C. Protein was extracted using high salt buffer and repeated (5×) freeze-thaw cycles. Nuclear protein DNA binding reactions were performed as described [11] in 20 μl containing 25 micrograms of protein extract and duplex oligonucleotide DNA binding elements for NF-κB (5′-GTTGACCCCTGAGAGGGAAAC-3′) and Stat3 (5′-CTAGGTAAAGGGCATTTAGCTAG-3′) oligonucleotides were synthesized. The probes for NF-κB and Stat3 were end labeled with gamma ^{32}P dATP with polynucleotide kinase. The binding reaction was performed for 20 min at room temperature and samples separated on a nondenaturing 5% PAGE gels with Tris-borate-EDTA buffer were utilized. Gels were dried. Bands were visualized and quantitated using a Molecular Imager FX (Biorad).

Statistical Analysis

Groups were analyzed using one-way analysis of variance (ANOVA). Statistical analysis of differences between groups was determined by post-hoc Student-Newman-Keuls test. Comparisons between final and initial laboratory values were analyzed using the paired Student's t test. Data are presented as means ± SEM. Statistical significance was defined as a P-value of less than 0.05. Statistical analysis was performed using commercially available software from Primer, McGraw-Hill, Inc.

TABLE 1

Physiologic and Laboratory Parameters of Swine Subjected to UHS Versus PUHS

	EBL (ml)	MAP (mm Hg)	Decrease in HCT
UHS	769 (60)*	86 (1.8)**	12.4 (1.3)***
PUHS	2187 (577)*	36 (0.3)**	22.5 (2.0)***

Mean (+/−SEM).
* $P < 0.01$.
** $P < 0.001$.
*** $P < 0.001$.

RESULTS

Physiologic and Laboratory Values

As anticipated, estimated blood loss (EBL) as a result of hemorrhage caused by the Grade V vascular liver injury was greater in the PUHS group (2187 ± 577 ml; Table 1) than in the UHS group (769 ± 60 ml, $P < 0.001$). The volume of blood lost during the splenectomy was not statistically significant between the sham, UHS, and PUHS groups (data not shown). The MAP throughout the course of the study in the PUHS group was 36 mm Hg compared with 86 mm Hg in the UHS group ($P < 0.001$). All sham animals were normotensive throughout the study. The starting hematocrit in the PUHS group was 29.1%, which decreased to 6.6% by the end of study, for an overall decrease of 22.5 ± 2.0. The starting hematocrit for the UHS group was 32%, which decreased to 20%, for an overall decrease in hematocrit of 12.4 ± 1.3. The difference in the change in hematocrit between PUHS (22.5) and UHS (12.4) was statistically significant ($P < 0.001$).

Liver IL-6 production is increased following uncontrolled HS in swine; the increase is proportional to the severity of shock. We previously demonstrated that IL-6 is produced in the livers of rodents subjected to controlled HS [11, 12], in which it was demonstrated to be essential for liver inflammation and focal hepatic necrosis [4]. To assess whether increased IL-6 production occurs in uncontrolled HS in swine and whether increased production is proportional to the severity of HS, we measured IL-6 mRNA in the livers of the sham, UHS, and PUHS groups by Q-RT-PCR.

IL-6 mRNA levels in the sham animals were similar to levels of IL-6 in the livers of normal healthy control swine (Fig. 1). Liver IL-6 mRNA production in UHS animals increased 8.2-fold vs normal control animals ($P < 0.01$) and 4.6-fold vs sham ($P < 0.05$). In PUHS animals, liver IL-6 mRNA levels increased 162-fold vs

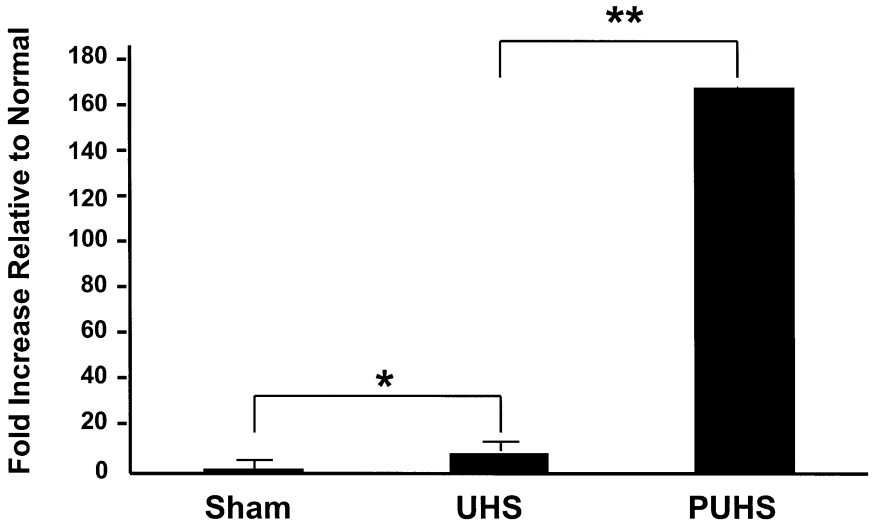

FIG. 1. IL-6 mRNA levels in liver of sham, UHS, and PUHS swine. IL-6 mRNA levels were quantitated in swine liver by Q-RT-PCR, as described in "Material and Methods," and standardized to levels detected in normal control liver ($n = 6$). Data presented are mean ± SEM of each group. Bars marked by one and two asterisks (*, **) differ significantly, $P < 0.01$ and $P < 0.001$, respectively.

normal controls ($P < 0.001$), 90-fold vs sham ($P < 0.01$), and 20-fold vs UHS ($P < 0.01$). These findings indicated that uncontrolled HS in swine resulted in increased liver IL-6 production and that the level of IL-6 production increased with severity of shock.

Liver NF-κB and Stat3 activity are increased following uncontrolled HS in swine; the increase is proportional to the severity of shock. We have previously demonstrated a 5 to 6-fold increase in liver production of IL-6 mRNA of rodents 4 h following controlled HS [11] that was accompanied by a 2 to 4-fold increase in NF-κB activity [13]. Administration of agents, such as iNOS inhibitors [11] and IL-6 [14], that reduced NF-κB activity inhibited liver production of IL-6 following HS. To determine whether NF-κB activation occurs in uncontrolled HS in swine and whether or not the level of NF-κB activation is proportional to the severity of shock, we measured NF-κB activity in the livers of each group of swine (Fig. 2). NF-κB activity in sham animals was similar to the level of activity observed in normal control animals (1.6-fold control, $P > 0.05$). NF-κB activity in UHS livers was increased vs normal controls (2.9-fold) and shams (2-fold) ($P < 0.05$ for each comparison). In PUHS livers, NF-κB activity was increased 3-fold vs normal controls and 1.9-fold vs sham ($P < 0.05$ for each comparison). The increase of NF-κB activity in shams was not statistically significant compared to normal controls.

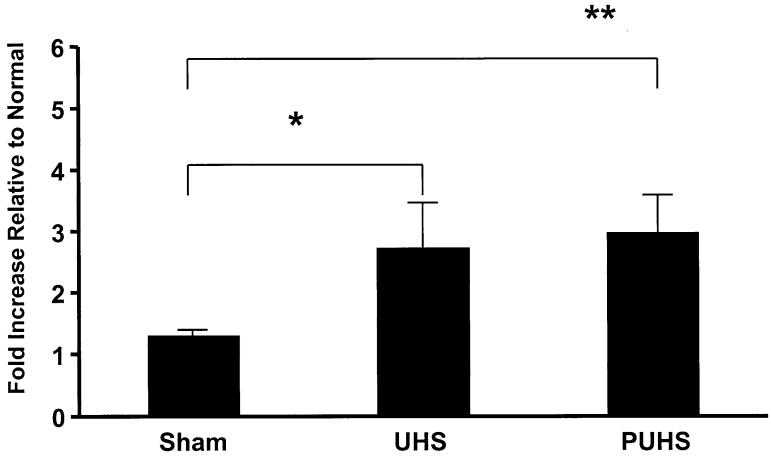

FIG. 2. Levels of NF-κB activity in livers of sham, UHS, and PUHS swine. NF-κB DNA-binding activity was assessed by EMSA using protein extracts of livers as described in "Material and Methods." The level of NF-κB activity was quantitated using a Molecular Imager FX (Biorad) and the level normalized to that present in extracts of normal liver. Data presented are the mean ± SEM of each group. Bars marked by one and two asterisks (*, **) differ significantly, both at $P < 0.05$.

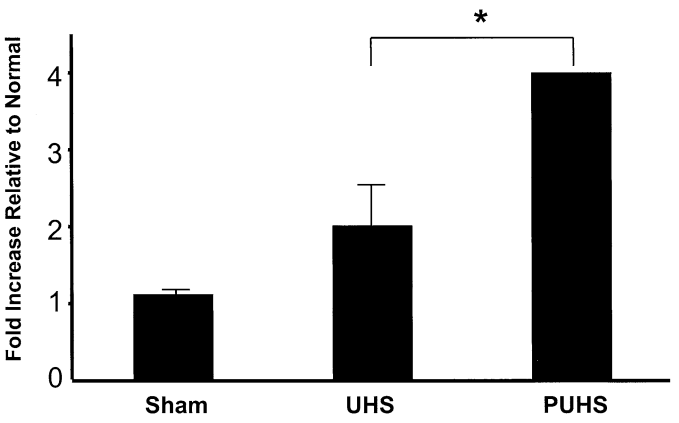

FIG. 3. Levels of Stat3 activity in livers of sham, UHS, and PUHS swine. Stat3 DNA-binding activity was assessed by EMSA using protein extracts of livers as described in "Material and Methods." The level of Stat3 activity was quantitated using a Molecular Imager FX (Biorad) and the level normalized to that present in extracts of normal liver. Data presented are the mean ± SD of each group. Bars marked by an asterisk (*) differ significantly, $P < 0.05$.

IL-6 binding to receptors on hepatocytes results in activation of Stat3, a transcription factor critical for the acute phase response [15]. We previously demonstrated that the activation of Stat3 in the liver of mice subjected to HS depended on the presence of IL-6 [4]. To determine whether the increased IL-6 mRNA production in the livers of shocked swine resulted in increased Stat3 activation and whether or not the level of Stat3 activation correlated with the severity of shock and the levels of IL-6 production, we measured Stat3 activity in whole liver by EMSA (Fig. 3). Although the 1.7-fold increase in Stat3 activity in UHS livers was not statistically different from normal control animals, Stat3 activity in the liver of PUHS animals was increased 5.3-fold vs normal controls and 3-fold vs UHS animals ($P < 0.05$ for each comparison). Therefore, similar to the findings for NF-κB, Stat3 activation is greatest in the group with the most severe shock.

Other Measures of Liver Injury: Liver Enzymes and Focal Liver Necrosis

Systemic evidence of diffuse liver injury was evident from elevation of the serum aspartate aminotransferase (AST) levels. Serum AST levels increased almost 3-fold in UHS animals from an initial mean value of 65 to a final mean value of 165.5 ($P < 0.001$). Serum AST levels increased eleven fold in PUHS animals from an initial mean value of 16 to a final mean value of 187 ($P < 0.05$). We did evaluate the extent of focal liver necrosis by H&E staining and found no significant differences at this early time point 2 h after injury in shams, UHS, or PUHS compared with normal animals reflected in the histology.

DISCUSSION

Uncontrolled hemorrhagic shock induced in swine by a Grade V vascular injury in the liver resulted in increased local IL-6 mRNA production that was proportional to the level severity of shock as assessed by EBL, MAP, and the magnitude of decrease in hematocrit. Similar to the findings for IL-6 mRNA production, the levels of NF-κB and Stat3 activation were greatest in the group with the most severe shock. These findings suggest that regional proinflammatory cytokine production results from and perpetuates a proinflammatory transcription factor cascade in swine models of uncontrolled hemorrhagic shock and indicate that this process is proportional to the severity of shock.

These results support the hypothesis that uncontrolled hemorrhagic shock in the swine model induces increased local production of proinflammatory cytokines, which in turn contribute to dysfunctional inflammation and end organ damage. Our findings in this and previous studies emphasize that serum levels of these cytokines are systemic "markers" of local injury. Organ damage is a local phenomenon that most likely occurs as a result of regional proinflammatory cytokine production. The data obtained from this large-animal model of uncontrolled hemorrhagic shock validates findings in rodent models of controlled hemorrhagic shock, in which it has been demonstrated that local production of endogenous IL-6 plays an important role in post resuscitation dysfunctional inflammation [2–5, 16].

We have focused our current investigation on the cytokine IL-6, because published reports [4] support an essential role for this cytokine in a murine model of controlled hemorrhagic shock. IL-6 is a 21-kDa cytokine produced by a variety of cell types including macrophages, endothelial cells, fibroblasts, hepatocytes, neutrophils, and T and B lymphocytes. IL-6 has both proinflammatory and antiinflammatory properties. IL-6 affects a variety of biological functions including immunoglobulin production, the acute phase response, and inflammation. Circulating IL-6 stimulates neutrophil production under stress conditions and is required for local production of CXC chemokines and neutrophil infiltration [17]. We have previously shown that IL-6 is increased in a rat model of controlled HS and that instillation of IL-6 into the rat lung results in neutrophil infiltration and lung injury [12, 14]. Furthermore, examination of mice deficient in IL-6 in a mouse model of controlled hemorrhagic shock revealed that local endogenous production of IL-6 is essential for organ inflammation and injury [4]. Our findings of increased local liver levels of IL-6 in uncontrolled HS in a large animal model that closely mimics HS in patients suggest that high circulating levels of IL-6 in HS patients may reflect high local levels of IL-6 production that contribute to increased organ damage.

Advantages and Limitations of Current Study

Our swine model of uncontrolled hemorrhagic shock fulfills a need for a model of HS that is uncontrolled and occurs in an animal close in size to humans. The need for such a model arises from the desire to validate, if possible, findings obtained in rodent HS models as well as to establish a model for preclinical testing of interventions designed to reduce dysfunctional inflammation and MODS.

Our study is limited by our decision to focus on only one proinflammatory cytokine and to examine only mRNA levels. Other well-known proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), were not examined in our current study. However, unlike IL-6, neither TNF-α nor IL-1β have been shown to be essential for organ inflammation and injury in HS. A disadvantage of working in the swine model is that there is a relative paucity of immunologic reagents. As a result, confirmatory studies at the protein level are limited.

A single point in time 2 h after injury was studied. No data on inflammation from later time points is given and survival information is not addressed. Because large animal studies are significantly more labor intensive and more costly than small animal studies, identification of the similar response to inflammation as seen in small animal models justifies further investigations of the immune response at different time points after injury.

An appropriate control to the PUHS group is lacking in this study design. Recognizing that inclusion of an appropriate control group made hypothermic and anemic by an albumin exchange transfusion is vital, subsequent studies that our laboratory has undertaken do include this crucial control group. We plan to examine differences in IL-6 mRNA and NF-κB and Stat3 activity in controls rendered hypothermic and anemic by an albumin exchange transfusion.

Swine models are being increasingly utilized in surgical studies, yet the inflammatory response of this species has not been well characterized at the molecular level. Although serum levels of IL-6 have been detected in hemorrhagic shock/trauma patients, the sources of their production and molecular events upstream are not completely understood. Although rodent models have provided new and additional insight to these events, determining whether or not molecular events described in rodents also occur in larger animals that can be sampled and evaluated more extensively and systematically than patients is an important step in understanding what occurs in hemorrhagic shock/trauma patients. In addition, characterization and use of a large-animal model to examine efficacy of interventions developed in rodents will provide further insight and confidence of efficacy before testing in humans.

CONCLUSION

Uncontrolled hemorrhagic shock induced in swine by a Grade V vascular injury in the liver resulted in increased local IL-6 mRNA production that was proportional to the level of severity of shock as assessed by EBL, MAP, and the magnitude of decrease in hematocrit. Similar to the findings for IL-6 mRNA, the levels of NF-κB and Stat3 activation were greatest in the group with the most severe shock. These findings suggest that regional proinflammatory cytokine production results from and perpetuates a proinflammatory transcription factor cascade in swine models of uncontrolled hemorrhagic shock and indicate that the magnitude of this process is proportional to the severity of shock.

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